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The Effects of Varying Dietary Protein, Calcium and Phosphorus in Starter Diets for Early Weaned PIgs

Wayne Eldon Schiefelbein

THE EFFECTS OF VARYING DIETARY PROTEIN, CALCIUM AND PHOSPHORUS IN STARTER DIETS FOR EARLY WEANED PIGS

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WAYNE ELDON SCHIEFELBEIN

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Richard C. Wahlstrom Date
Thesis Adviser

Joe A. Minyard Date
Head, Animal Science Department

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INTRODUCTION

Genetic selection by swine producers the past 10 to 15 years has resulted in faster growing, heavier muscled pigs. In addition, confinement rearing has placed an increased emphasis upon skeletal development and bone formation.

Recent research conducted at Ohio State University indicated that growing swine responded to increasing dietary protein levels if both calcium and phosphorus are also correspondingly increased but maintained at approximately a 1.3:1 ratio. This and other research has led some investigators to believe that the National Research Council's requirements for protein, calcium and phosphorus may not be adequate to support maximum growth and development.

Much attention has been given to the nutritional requirements of growing and finishing swine in recent years. Previous research has shown that maximum bone formation and muscle development occur during the first 12 weeks of life in swine. The purpose of the research reported herein was to investigate the effect of dietary protein levels and varying levels of calcium and phosphorus on performance of young weaned pigs. Three trials were conducted to study different levels of these three nutrients in starter diets. Parameters analyzed were average daily gain, feed consumption, feed efficiency, bone ash, bone weight, serum calcium and serum phosphorus.

REVIEW OF LITERATURE

Function and Requirement of Protein

According to Lehninger (1975), proteins are high molecular weight molecules formed from low molecular weight compounds called amino acids. Since they are fundamental in all aspects of cell structure and function, proteins are the most abundant organic molecule in cells and are found in every part of every cell. Thus a liberal and continuous supply of protein is needed in the diet for growth and repair of organs and soft tissue of the body (Maynard and Loosli, 1962).

The National Research Council (NRC, 1973) lists dietary protein requirements of 22 and 18% for 5- to 10-kg and 10- to 20-kg pigs, respectively. Meade *et al*. (1969) demonstrated slower and less efficient gains from 15% protein starter diets than 18% diets when fed during a period from 5.9 to 23.5 kg. No improvement in performance was observed when feeding diets containing more than 18% protein.

Jensen *et al*. (1957) reported pigs required at least 17% dietary protein during the period from 2 to 8 weeks of age. They also found gain/feed improved markedly as level of dietary protein was increased from 10 to 17%.

Rutledge *et al*. (1961) reported 3- to 9-week old pigs required 20% protein for maximum nitrogen retention and gain during early stages of the growth period, but suggested a lower level was adequate for maximum gains during latter stages of the trials. Feed utilization tended to improve as dietary protein increased from 15 to 30%.

Some apparent discrepancies in minimum protein requirements are due in part to differences among rations fed in the studies cited. Crampton and Ness (1954) noted best overall performance at 30% dietary protein level using diets containing a poor balance of amino acids. Jensen et al. (1957) reported a 17% protein diet with balanced amino acids provided adequate performance. Thus the level and balance of essential amino acids may be more important than percentage of crude protein in diets of young pigs.

Function and Requirement of Calcium and Phosphorus

More than 99% of body calcium and 75% of total phosphorus can be found in bones and teeth. Bone is the reservoir for both of these minerals and the calcium and phosphorus concentration found in the trabecular portion of bone is in equilibrium with that of body fluids and other tissues (Hays and Swenson, 1977).

Both macrominerals serve as structural framework. Calcium is an important constituent for formation of thrombin, muscle contraction and intracellular environment. Maynard and Loosli (1962) stated phosphorus has more functions than any other element and is found in every cell of the body. According to Hays and Swenson (1977), phosphorus is an important part of metabolic processes and buffering in body fluids and almost every form of energy transfer inside living cells involves forming or breaking high energy bonds that link oxides of phosphorus compounds.

Much of the phosphorus in plant seeds is in the form of phytin, the calcium and magnesium salts of the hexaphosphate ester of inositol. In order for the phytate phosphorus to be utilized by pigs it must be hydrolyzed from the molecule which is accomplished by the enzyme phytase. Considerable variability in phytase content of feedstuffs does exist and seldom are the conditions for phytase activity optimum for any period of time. This makes phytate phosphorus an undependable source for pigs. On the other hand, non-phytin phosphorus is quite available to pigs. Therefore, adjustments in the dietary level of total phosphorus is needed to attain adequate levels of this mineral available for both bone and soft tissue development.

A precise estimate of the availability of total plant phosphorus is difficult because of the variability of feedstuffs. From the results of digestibility studies, Bayley and Thomson (1969) estimated that only 20 to 30% of the total plant phosphorus in a corn-soybean meal mixture is available to the pig.

Early studies were conducted by Wintrobe (1939) on baby pigs fed synthetic milk diets. He reported satisfactory growth rate when calcium treatments consisted of 0.94% of the total solids portion of the diet. In a similar study, Johnson _et al_. (1948) found a 0.91% calcium value was required for adequate growth.

Zimmerman _et al_. (1960) conducted studies on baby pigs 2 to 6 weeks of age fed high milk diets. Calcium levels ranged from 0.52 to 1.05% and were used in combination with either 0.52 or 0.70% phosphorus. They reported 0.70% calcium adequate for maximum rate and efficiency of gain and noted a growth depression when levels greater than 0.80% were fed. Bone calcification was greater at higher calcium levels.

In a similar study conducted by Miller et al. (1962) on baby pigs, maximum rate and efficiency of gain were observed at a 0.60% calcium level. Normal serum calcium levels were reported at 0.80% dietary calcium.

Rutledge et al. (1961) examined the calcium requirement of 3- to 9-week old weaned pigs fed a practical type ration. Diets contained calcium levels of 0.40, 0.60, 0.80, or 1.0% and a constant 0.60% phosphorus level. Highly significant linear trends toward increased ash, calcium content, and breaking strength of bone resulted from increased levels of dietary calcium. They suggested a 0.80% dietary calcium is near the minimum requirement for normal bone development.

Newman et al. (1967) fed weanling pigs diets calculated to contain 0.20, 0.40, 0.60, and 0.80% calcium and 0.45% phosphorus. Feed intake and average daily gain increased linearly up to 0.60% dietary calcium. Maximum bone density was attained in pigs receiving 0.60% calcium whereas breaking strength was increased linearly through the 0.80% level.

Miller et al. (1964) conducted phosphorus studies with baby pigs fed synthetic milk diets. Phosphorus levels which varied from 0.20% to 0.80% were evaluated with a calcium level of 0.80%. They concluded a dietary phosphorus level of 0.60% appeared adequate for normal growth and economy of feed utilization. To maintain normal concentrations of serum calcium and phosphorus and provide adequate rate of skeletal growth, a 0.50% dietary phosphorus level was required. The authors also reported 0.60% phosphorus as a minimum to obtain maximum strength of bone. Zimmerman et al. (1963) obtained optimal performance with 3- to 7-week old pigs when their high milk-product diet contained 0.60% phosphorus.

Researchers are aware of the relationship between calcium and phosphorus and the effect that ratio and level of these minerals have on response criteria. Combs et al. (1962) conducted trials on early weaned pigs from 2- to 7-weeks of age fed a fortified corn-soybean meal diet. They fed phosphorus levels varying from 0.24 to 0.72% with calcium: phosphorus ratios of 1.2:1 and 2:1, and phosphorus levels of 0.40 to 0.48% at ratios of 0.9:1, 1.2:1, and 1.5:1. They established that 0.44% dietary phosphorus at a 0.9:1 calcium:phosphorus ratio was necessary for optimum results when weight gain, feed efficiency, percent ash, radio graphs of the fibula and femur and length of femur were used as response criteria.

Zimmerman et al. (1963) fed calcium levels of 0.50, 0.60, 0.80, and 0.95% and phosphorus levels of 0.40, 0.50. 0.60, and 0.70% in all possible combinations to pigs 2- to 7-weeks of age. The results suggested a maximum calcium level of 0.80% and minimum phosphorus level of 0.60% for maximum performance and adequate skeletal development. They observed that low phosphorus levels and high calcium levels and calcium: phosphorus ratios of 1.6:1 or wider decreased rate of gain.

Coalson et al. (1972) conducted two experiments using 3- to 9-week old pigs to evaluate the effects of various levels of calcium and phosphorus. Purified diets in trial 1 contained 0.27, 0.57, 0.95, and 1.25% calcium and 0.14, 0.44, 0.73, and 1.05% phosphorus, respectively.

In trial 2, diets contained 0.37, 0.57, 0.78, and 0.95% calcium and 0.28, 0.44, 0.61, and 0.73% phosphorus, respectively. The researchers observed growth rates were increased linearly as the dietary levels of calcium and phosphorus were increased. Diets containing 0.95% calcium and 0.73% phosphorus supported best gain, feed efficiency and optimal skeletal development as measured by bone weight, diameter, ash, calcium, and phosphorus content.

Protein, Calcium and Phosphorus Relationships

Recent research conducted by Reinhard et al. (1976) suggested growing swine responded to increasing dietary protein if both calcium and phosphorus are correspondingly increased. In two trials they fed corn-soybean meal diets formulated to contain from 14 to 22% dietary protein while maintaining calcium and phosphorus at 0.65 and 0.50%, respectively. Generally there was an increase in daily gains as protein level increased from 14 to 16 or 18%, but there was a slight decrease at higher levels. They also noted a reduction in feed intake in both experiments at the higher protein levels. The researchers initiated another trial and fed 16 and 22% protein diets containing 0.65/0.50%, 0.90/0.70%, and 1.15/0.90% dietary calcium and phosphorus, respectively, at each protein level. Elevating calcium and phosphorus in either 16 or 22% protein diets resulted in increased growth, serum phosphorus, and bone ash.

Fammatre et al. (1977) conducted an experiment to evaluate the effects of two levels of dietary calcium and phosphorus (0.90/0.70% or

0.65/0.50%, respectively) in 18% protein diets for growing swine. They reported increased gain and feed intake at the higher mineral levels. Increasing the calcium and phosphorus level also resulted in an increase in bone ash and tendancy for increased serum phosphorus values.

Although more attention has been devoted to the effects of varying levels of protein, calcium and phosphorus for growing swine in recent years, Brown et al. (1972) demonstrated maximum bone formation occurs in the first 12 weeks of life in swine. This is the same period that maximum muscle development occurs.

MATERIALS AND METHODS

Three trials were conducted with 144 pigs in each of trials 1 and 2 and 96 pigs in trial 3. Pigs used were crossbreds of Duroc, Yorkshire, and Hampshire breeding and weighed between 7.5 and 10 kg initially. They ranged in age from 4 to 6 weeks and were all farrowed at the South Dakota State University Swine Unit. Each 28 day trial consisted of four replications of six dietary treatments.

All male pigs were castrated at approximately 14 days of age. Pigs were weighed periodically from 28 days of age and put on test a replication at a time to insure adequate pig numbers and more uniformity of weights. The experimental animals were stratified to treatments according to weight and sire. Sex was not considered for allotment. The pigs were not creep fed and when they reached the correct weight, were removed from their dam and put directly on experimental diets.

The experiments were housed in the environmentally controlled swine lab of the Animal Science Complex. Air temperature ranged from 27° C initially to about 22° C as the trials were terminated.

Each treatment group was confined in 1.21 x 1.75 m pens. Every pen was provided with a self feeder and nipple waterer. The floors contained approximately 67% solid surface area and the remaining floor area as expanded metal covering a pit. The pens were not bedded, but were washed every other day with a pressure hose.

Corn-soybean meal diets were processed through a conventional hammer mill with a 4.8 mm screen. Vitamin and antibiotic premixes were preweighed and mixed with corn as a carrier before being added to the other ingredients in a twin spiral vertical mixer at the Central Feed Unit. An anthelmentic was added to the diets in trials 2 and 3 for protection against internal parasites. The protein levels were varied by altering the soybean meal:corn ratio and the various combinations of calcium and phosphorus were obtained using limestone and dicalcium phosphate. Proximate analysis and calcium and phosphorus analysis for all experimental diets are shown in appendix tables 1-3.

<u>Trial 1</u>. <u>Increasing Dietary Protein and Mineral With Calcium:Phosphorus Ratio At 1.3:1</u>.

The first trial was started July 5, 1977 and terminated August 17, 1977. One hundred forty-four pigs averaging approximately 8.5 kg were allotted to six treatments with four replications per treatment. Respective percentages of protein, calcium, and phosphorus for the six treatments were as follows:

Ration 1. 18%, 0.80 and 0.60%

Ration 2. 18%, 1.05 and 0.80%

Ration 3. 18%, 1.30 and 1.0%

Ration 4. 21%, 0.80 and 0.60%

Ration 5. 21%, 1.05 and 0.80%

Ration 6. 21%, 1.30 and 1.0%

High analysis soybean oil meal (48%) was used as the protein supplement. Composition of experimental diets and calculated analyses of protein, calcium, and phosphorus are shown in table 1. Blood samples were collected at the beginning and termination of trial 1.

TABLE 1. PERCENTAGE COMPOSITION OF EXPERIMENTAL DIETS.

TRIAL 1

Ingredients	Dietary Treatments 1	2	3	4	5	6
Ground yellow corn	72.91	71.42	70.04	65.45	63.96	62.47
Soybean oil meal (48%)	24.00	24.30	24.50	31.60	31.90	32.20
Dicalcium phosphate	1.15	2.25	3.34	1.00	2.10	3.19
Limestone	1.24	1.33	1.42	1.25	1.34	1.44
Trace mineralized salt[a]	.4	.4	.4	.4	.4	.4
Vitamin-antibiotic premix[b]	.3	.3	.3	.3	.3	.3
Calculated analysis						
Protein, %	18.01	18.02	17.99	20.99	21.00	21.02
Calcium, %	.86	1.05	1.30	.80	1.05	1.30
Phosphorus, %	.60	.80	1.0	.60	.80	1.0

[a]Composition shown in appendix table 4.

[b]Composition shown in appendix table 5.

Trial 2. Increasing Dietary Protein and Phosphorus With Calcium Level Constant.

Trial 2 was initiated October 6, 1977 and terminated November 18, 1977. The trial utilized 144 pigs averaging 9.1 kg in six treatments replicated four times. Levels of protein, calcium and phosphorus, respectively, were as follows:

Ration 1. 18%, 0.80 and 0.60%

Ration 2. 18%, 0.80 and 0.80%

Ration 3. 18%, 0.80 and 1.0%

Ration 4. 21%, 0.80 and 0.60%

Ration 5. 21%, 0.80 and 0.80%

Ration 6. 21%, 0.80 and 1.0%

Several analyses of high protein soybean oil meal (48%) showed discrepancies from guaranteed analysis for that supplement, therefore a lower protein soybean meal (44%) was used. Experimental diets and calculated analyses are shown in table 2. Blood samples were collected at the beginning and termination of experiment 2. One pig per pen was sacrificed and front feet extracted for bone analysis.

Trial 3. Increasing Dietary Protein and Mineral Levels With Calcium: Phosphorus Ratio At 1.25:1.

The third trial was conducted from July 4, 1978 to August 14, 1978. Ninety six pigs averaging 8.6 kg were assigned to six treatments. Percentage of dietary protein, calcium, and phosphorus, respectively, were as follows:

TABLE 2. PERCENTAGE COMPOSITION OF EXPERIMENTAL DIETS.

TRIAL 2

Ingredients	Dietary Treatments					
	1	2	3	4	5	6
Ground yellow corn	71.07	70.44	69.79	62.91	62.26	61.52
Soybean oil meal (44%)	25.80	25.90	26.00	34.10	34.20	34.40
Dicalcium phosphate	1.18	2.26	3.35	1.03	2.12	3.20
Limestone	1.25	.7	.16	1.26	.72	.18
Trace mineralized salt[a]	.4	.4	.4	.4	.4	.4
Vitamin-antibiotic premix[b]	.3	.3	.3	.3	.3	.3
Calculated analysis						
Protein, %	18.01	17.99	17.98	21.00	20.99	21.02
Calcium, %	.80	.80	.80	.80	.80	.80
Phosphorus, %	.60	.80	1.00	.60	.80	1.00

[a]Composition shown in appendix table 4.

[b]Composition shown in appendix table 5.

Ration 1. 18%, 0.75 and 0.60%

Ration 2. 18%, 1.0 and 0.80%

Ration 3. 18%, 1.25 and 1.0%

Ration 4. 22%, 0.75 and 0.60%

Ration 5. 22%, 1.0 and 0.80%

Ration 6. 22%, 1.25 and 1.0%

Composition of experimental diets and calculated analyses are shown in table 3.

Criteria of response for all trials included average daily gain, feed consumption, and feed efficiency. The one pig that died while on test was taken to the Veterinary Diagnostic Laboratory for post-mortem examination. Average daily gain and feed consumption were calculated only for those pigs finishing the experiment. Feed consumption data were corrected by subtracting an average value of the feed consumed to the time of death.

Approximately 15 ml of blood was collected from each experimental animal at the beginning of trials 1 and 2. At the conclusion of these trials another blood sample was taken, after 4 hours of fasting. The blood was drawn from the cranial vena cava and deposited into centrifuge tubes in which the whole blood was refrigerated and allowed to clot. The samples were centrifuged for 10 minutes at 1100 rpm and the serum extracted and frozen until analyzed.

Both serum calcium and phosphorus were determined and reported in mg per 100 ml of serum. Calcium levels of the serum were determined by atomic absorption spectrophotometry with a Perkin-Elmer spectrophotometer 303. Phosphorus levels were determined colorimetrically with an

TABLE 3. PERCENTAGE COMPOSITION OF EXPERIMENTAL DIETS.

TRIAL 3

	Dietary Treatments					
Ingredients	1	2	3	4	5	6
Ground yellow corn	70.54	69.06	67.59	59.31	57.83	56.41
Soybean oil meal (44%)	26.50	26.80	27.10	37.90	38.20	38.45
Dicalcium phosphate	1.16	2.26	3.35	.96	2.06	3.15
Limestone	1.10	1.18	1.26	1.13	1.21	1.29
Trace mineralized salt[a]	.4	.4	.4	.4	.4	.4
Vitamin-antibiotic premix[b]	.3	.3	.3	.3	.3	.3
Calculated analysis						
Protein, %	18.01	18.01	18.00	22.01	22.01	22.00
Calcium, %	.75	1.00	1.25	.75	1.00	1.25
Phosphorus, %	.60	.80	1.00	.60	.80	1.00

[a]Composition shown in appendix table 4.

[b]Composition shown in appendix table 5.

Evelyn photoelectric colorimeter by the procedure of Fisk and Subbarow (1925) as outlined by Hawk et al. (1947). Generally, only one analysis of both calcium and phosphorus was determined for each blood sample. To eliminate contamination a non-phosphate soap was used for cleaning lab equipment and double distilled water was used for all rinsing. Following rinsing, all glassware was acid treated.

One pig from each pen in trial 2 was sacrificed and metacarple bones from both front feet removed and identified for determination of bone weight and ash. The bones were boiled for 30 minutes after which the excess soft tissue and cartilage was scraped off. After drying 24 hours at 100° C in an oven, dry weights were recorded for each bone.

A one-eighth inch hole was drilled in each end of every bone. They were extracted in ethyl-ether for 24 hours through the use of a side-arm extractor. The samples were dried for 2 hours and crushed. They were ashed at 600° C for 12 hours and bone ash and crucible were weighed while still warm to insure a moisture-free weight.

The data collected were analyzed statistically by least squares analysis of variance as outlined by Steel and Torrie (1960). Tukey's "w" procedure was used to determine significant differences between main effects and treatments when significant differences were obtained within trials.

RESULTS AND DISCUSSION

Trial 1. Effects of Increasing Dietary Protein and Mineral With Calcium:Phosphorus Ratio at 1.3:1.

The performance data from the first trial are summarized in table 4. The statistical analyses for this trial are presented in appendix tables 6-9.

Pigs fed the 21% protein diets had a feed to gain ratio of 1.72 which was significantly ($P<.01$) more efficient than the ratio of 1.87 of pigs receiving 18% dietary protein. There were no significant differences in average daily gain or feed intake among treatments. However, pigs fed the high protein diet with calcium and phosphorus levels of 1.3 and 1.0%, respectively, gained approximately 12% faster than pigs fed the other diets. Reinhard et al. (1976) reported a similar growth response with corresponding increasing dietary protein, calcium and phosphorus levels with growing swine. Increasing the mineral levels above the NRC recommended levels did not depress feed intake or performance as earlier reported by Combs and Wallace (1962).

Blood serum calcium and phosphorus were determined on all experimental animals. The results obtained from serum calcium and phosphorus analysis are shown in table 5. Serum values obtained were somewhat higher than those reported by Reinhard et al. (1976) and Fammatre et al. (1977) for growing swine, but were within the established values of 9-11 mg % serum calcium and phosphorus reported by Hays and Swenson (1977). Initial and termination serum calcium ranged from 10.47-10.70

TABLE 4. EFFECT OF VARYING DIETARY PROTEIN, CALCIUM AND PHOSPHORUS ON FEEDLOT PERFORMANCE. TRIAL 1

	Nutrient %	Dietary treatments (protein and mineral)						Means of main effects				
	C.P.	18			21			18	21	...	...	...
	Ca	0.80	1.05	1.3	0.80	1.05	1.3	...	...	0.80	1.05	1.30
Item	P	0.60	0.80	1.0	0.60	0.80	1.0	...	...	0.60	0.80	1.0
Final wt., kg		20.45	20.77	20.32	20.36	20.77	22.32	20.51	21.11	20.40	20.78	21.24
Daily gain, kg		0.41	0.42	0.41	0.41	0.42	0.47	0.41	0.43	0.41	0.42	0.44
Daily feed consumption, kg		0.75	0.80	0.75	0.71	0.71	0.81	0.77	0.74	0.73	0.75	0.78
Feed/gain ratio[a]		1.84	1.90	1.87	1.74	1.68	1.70	1.87	1.71	1.77	1.79	1.78

[a]Significant ($P<.01$) protein effect (1.71 for 21% protein vs. 1.87 for 18% protein).

TABLE 5. EFFECT OF VARYING DIETARY PROTEIN, CALCIUM AND PHOSPHORUS ON SERUM CALCIUM AND PHOSPHORUS. TRIAL 1

Item	Nutrient %	Dietary treatments (protein and mineral)						Means of main effects				
	C.P.	18			21			18	21	...	...	...
	Ca	0.80	1.05	1.3	0.80	1.05	1.3	...	...	0.80	1.05	1.30
	P	0.60	0.80	1.0	0.60	0.80	1.0	...	...	0.60	0.80	1.0
Serum calcium, mg/100 ml												
Initial		10.47	10.58	10.70	10.61	10.63	10.45	10.59	10.57	10.54	10.61	10.58
Termination		10.38	10.50	10.51	10.61	10.56	10.37	10.46	10.52	10.50	10.53	10.44
Difference		-0.08	-0.09	-0.19	0.01	-0.07	-0.08	-0.12	-0.04	-0.04	-0.08	-0.14
Serum phosphorus, mg/100 ml												
Initial		9.72	9.63	9.71	9.58	9.47	9.64	9.68	9.56	9.65	9.55	9.67
Termination[a]		10.53^{b}	10.35^{bc}	9.64^{cd}	9.05^{d}	10.13^{bc}	10.16^{bc}	10.17	9.75	9.79	10.24	9.88
Difference		0.81^{e}	0.77^{e}	-0.07^{ef}	-0.53^{f}	0.66^{e}	0.53^{ef}	0.49	0.19	0.14	0.72	0.21

[a] Significant (P<.05) protein effect (10.17 mg % for 18% protein vs. 9.75 mg % for 21% protein).

[bcd] Means with different superscripts are significantly different (P<.01).

[ef] Means with different superscripts are significantly different (P<.05).

and 10.37-10.61 mg %, respectively. There were no differences (P<.05) between initial and terminal serum calcium and no differences among treatments. According to Maynard and Loosli (1962), blood calcium originates from the diet but the level in the serum is under the influence of several physiological factors, the most important of which may be the hormone secreted by the parathyroid gland. Irving (1973) more recently stated that in the normal animal there is a suggestion that the hormone calcetonin secreted by the thyroid gland may play an important role with parathormone in maintaining serum calcium homeostasis in the animal body.

At the conclusion of trial 1, pigs receiving the higher protein (21%) diets had average serum phosphorus value of 9.75 mg % which was lower (P<.05) than the average 10.17 mg % serum phosphorus of those pigs receiving lower protein (18%) diets, suggesting higher dietary phosphorus levels may be necessary at increased protein levels. This may be further illustrated by the lower serum phosphorus concentration observed in pigs fed the 21% protein diet with 0.80% calcium and 0.60% phosphorus. The serum phosphorus levels of pigs fed this diet was 9.05 mg % which was significantly lower (P<.05) than that of pigs on all other treatments with the exception of the 9.64 mg % of pigs fed 18% protein with 1.3% calcium and 1.0% phosphorus. This treatment response of 9.64 mg % was lower (P<.05) than 10.53 mg % serum phosphorus of pigs receiving 0.80% calcium and 0.60% phosphorus at the 18% protein level; however, this did not appear to be related to growth performance of the pigs. Fammatre *et al*. (1976) found that the availability of phosphorus was lower in higher protein diets resulting in lower serum concentrations of this nutrient. Maynard and Loosli (1962) stated a rapid rate of growth which is accompanied by normal levels of calcium and phosphorus in the blood is highly indicative of adequate skeletal development and may be necessary to determine the mineral requirements of pigs.

Differences between termination and initial serum concentrations were calculated and analyzed. Initial serum phosphorus did not differ among treatments and ranged from 9.47 to 9.72 mg %. Blood serum of pigs fed the 21% protein diet containing 0.80/0.60% calcium:phosphorus decreased in phosphorus content by 0.53 mg % and that of pigs fed the 18% protein diet with 1.3% calcium and 1.0% phosphorus decreased 0.07 mg % in phosphorus content. All other treatments resulted in increased serum phosphorus of 0.53 to 0.81 mg %. Variations between initial and final serum phosphorus values were significant ($P<.05$) among treatments receiving 21% protein, 0.80% calcium and 0.60% phosphorus and those of 21% protein, 1.05% calcium and 0.80% phosphorus, 18% protein, 0.80% calcium and 0.60% phosphorus and 18% protein, 1.05% calcium and 0.80% phosphorus.

Trial 2. Effects of Increasing Dietary Protein and Phosphorus With Calcium Level Constant.

The performance data for trial 2 are shown in table 6. The statistical data for the trial are presented in appendix tables 10-13.

Calcium and phosphorus ratio did not appear to have an effect on performance. The ratios were within accepted ranges of approximately

TABLE 6. EFFECT OF VARYING DIETARY PROTEIN AND PHOSPHORUS ON FEEDLOT PERFORMANCE. TRIAL 2

	Nutrient %	Dietary treatments (protein and mineral)						Means of main effects				
	C.P.	18			21			18	21	...	...	...
	Ca	0.80	0.80	0.80	0.80	0.80	0.80	...	...	0.80	0.80	0.80
Item	P	0.60	0.80	1.0	0.60	0.80	1.0	...	...	0.60	0.80	1.0
Final wt., kg		20.38	21.21	20.65	20.58	21.16	21.70	20.75	21.15	20.48	21.18	21.18
Daily gain, kg		0.40	0.43	0.41	0.41	0.43	0.45	0.42	0.43	0.41	0.43	0.43
Daily feed consumption, kg		0.74	0.78	0.78	0.71	0.74	0.71	0.76	0.72	0.72	0.75	0.75
Feed/gain, ratio[a]		1.84[b]	1.80[bc]	1.89[b]	1.72[de]	1.72[de]	1.65[cd]	1.85	1.70	1.79	1.76	1.78

[a]Significant (P<.01) protein effect (1.70 for 21% protein vs. 1.85 for 18% protein).

[bcde]Means with different superscripts are significantly different (P<.01).

1.3:1 as suggested by Doige et al. (1975) and Pond et al. (1975) to approximately 0.80:1.0 as reported by Combs et al. (1962).

As indicated in the first trial, feeding pigs diets containing 21% protein with 1.0% phosphorus resulted in the fastest gains. Gains of pigs fed this higher protein diet increased from 0.41 to 0.43 to 0.45 kg daily as dietary phosphorus increased from 0.60 to 0.80 to 1.0 percent. Day and McCollum (1939) presented evidence on the needs of phosphorus for soft tissue deposition. In view of the trend towards increasing muscle deposition and performance, there is a possibility of greater need for dietary protein and phosphorus. Cromwell et al. (1970) demonstrated that slight phosphorus deficiencies could result in decreased gains, while in a more recent study (Cromwell et al., 1972) they reported decreases in dietary calcium generally did not cause a decrease in performance.

Feeding pigs 21% dietary protein resulted in a feed to gain ratio of 1.70 which was more efficient (P<.01) than the 1.85 ratio of pigs fed 18% protein diets. Rutledge et al. (1961) noted similar results with 3- to 9-week old pigs fed varying levels of protein. They observed that even though lower protein levels were adequate for maximum gains, during latter stages of the trials feed utilization tended to improve as dietary protein increased from 15 to 30%.

The feed to gain ratio of 1.65 of pigs fed the 21% protein diet containing 1.0% phosphorus was lower (P<.01) than the ratios of 1.84, 1.80 and 1.89 of pigs fed 18% protein diets containing 0.60, 0.80 or 1.0% phosphorus, respectively. Pigs receiving diets formulated to contain

21% protein and 0.60% or 0.80% phosphorus required less feed per unit of gain (P<.01) than did those pigs fed dietary treatments of 0.60 or 1.0% phosphorus in 18% protein diets.

Blood samples were obtained from each experimental animal at the beginning and termination of the trial and analyzed for serum calcium and phosphorus. The results are presented in table 7. A significant mineral effect (P<.01) was noted for termination serum calcium concentration. Averaging over protein levels, phosphorus diets of 0.60, 0.80 and 1.0% resulted in serum calcium levels of 10.16, 9.95 and 9.74 mg %, respectively. Also, although not significant, phosphorus levels in the serum increased linearly from 10.37 to 10.56 to 10.84 mg % with the increasing levels of phosphorus in the diet. This inverse relationship between serum calcium and phosphorus in the pig is in agreement with earlier work reported by several researchers (Miller _et al_., 1964; Harmon _et al_., 1967; and Cromwell _et al_., 1970).

Some blood phosphorus patterns that were noted in trial 1 were also observed in trial 2. Pigs fed 21% protein rations with 0.60, 0.80 and 1.0% phosphorus resulted in serum phosphorus concentrations of 9.80, 10.57 and 10.95 mg %, respectively. Supplementing the high protein diets with 1.0% dietary phosphorus raised the serum phosphorus concentration to the level of pigs fed the basal diet (18% protein and 0.60% phosphorus). As in trial 1, pigs fed the 21% protein diet with 0.60% phosphorus had the lowest serum phosphorus concentration. The blood phosphorus of pigs fed this treatment was significantly lower (P<.05) than those receiving diets containing 1.0% dietary phosphorus at both protein levels and the

18% protein diet of 0.60% phosphorus. When the differences between termination and initial serum phosphorus concentrations were determined and analyzed, a similar pattern to that of termination blood phosphorus was observed.

Results of the bone analyses are also listed in table 7. The average final weights of the pigs used for bone analysis were 18.1, 18.6, 18.6, 18.5, 18.7 and 19.4 kg, respectively as the treatments are listed in table 7. Those pigs fed the 21% protein diets averaged 0.5 kg heavier body weights at the time of slaughter than those fed 18% protein diets. However, the average bone weight of 4.11 g of pigs fed 18% protein diets was heavier (P<.01) than the 3.95 g average of pigs receiving 21% protein diets. These results indicate an increase of dietary phosphorus may be necessary to match skeletal development with increased performance when higher protein diets are fed. The data obtained on percent bone ash did not substantiate the bone weight results in that pigs receiving 21% protein diets had bone ash values averaging 52.8% which was slightly greater (P<.05) than the 52.5% ash average of pigs that were getting 18% dietary protein. Nelson and Walker (1964) suggested that percentage bone ash is one of the most sensitive criteria for evaluating the availability of phosphorus, more so than weight gains, as it is little affected by other dietary variations that influence growth.

Averaging over protein levels, a similar phosphorus level effect was observed for both bone weight and percent bone ash. Bone weights were 3.90, 4.15 and 4.04 g and bone ash values were 51.5, 53.3 and 53.2%

TABLE 7. EFFECT OF VARYING DIETARY PROTEIN AND PHOSPHORUS ON BLOOD AND BONE ANALYSIS. TRIAL 2

Item	Nutrient %	Dietary treatments (protein and mineral)						Means of main effects				
	C.P.	18			21			18	21	...	...	...
	Ca	0.80	0.80	0.80	0.80	0.80	0.80	...	...	0.80	0.80	0.80
	P	0.60	0.80	1.0	0.60	0.80	1.0	...	...	0.60	0.80	1.0
Serum calcium, mg/100 ml												
Initial		10.03	10.16	10.10	10.07	9.87	9.98	10.10	9.97	10.05	10.02	10.04
Termination[a]		10.26	9.96	9.71	10.06	9.94	9.77	9.98	9.92	10.16	9.95	9.74
Difference[b]		0.23	-0.19	-0.39	-0.01	0.07	-0.22	-0.11	-0.05	0.11	-0.06	-0.30
Serum phosphorus, mg/100 ml												
Initial		9.72	9.94	9.54	9.81	9.86	9.91	9.73	9.86	9.76	9.90	9.73
Termination		10.93[c]	10.55[cd]	10.73[c]	9.80[d]	10.57[cd]	10.95[c]	10.74	10.44	10.37	10.56	10.84
Difference		1.22[e]	0.61[ef]	1.19[e]	-0.01[f]	0.71[ef]	1.03[ef]	1.01	0.58	0.61	0.66	1.11
Bone analysis												
Bone weight, g[gh]		4.04[k]	4.02[k]	4.26[j]	3.76[i]	4.28[j]	3.81[i]	4.11	3.95	3.90	4.15	4.04
Percent bone ash[lm]		51.1[p]	52.6[n]	53.7[o]	51.8[np]	54.0[o]	52.7[n]	52.5	52.8	51.5	53.3	53.2

[a]Significant (P<.01) mineral effect (0.60% phosphorus at 10.16 mg % vs. 1.0% phosphorus level at 9.74 mg %).

[b]Significant (P<.05) mineral effect (0.60% phosphorus at 0.11 mg % vs. 1.0% phosphorus at -0.30 mg %).

[cd]Means with different superscripts are significantly different (P<.05).

TABLE 7. EFFECT OF VARYING DIETARY PROTEIN, CALCIUM AND PHOSPHORUS ON BLOOD AND BONE ANALYSIS. TRIAL 2 (CONTINUED)

[ef]Means with different superscripts are significantly different (P<.05)

[g]Significant (P<.01) protein effect (4.11 g for 18% protein vs. 3.95 g for 21% protein).

[h]Significant (P<.01) mineral effect (3.90 vs. 4.15 vs. 4.04 g for 0.60, 0.80 and 1.0% dietary phosphorus, respectively).

[ijk]Means with different superscripts are significantly different (P<.01).

[l]Significant (P<.05) protein effect (52.8% for 21% protein vs. 52.5% for 18% protein).

[m]Significant (P<.01) mineral effect (51.5% at 0.60% phosphorus vs. 53.3 and 53.2% ash at 0.80 and 1.0% dietary phosphorus, respectively).

[nop]Means with different superscripts are significantly different (P<.05).

for pigs fed 0.60, 0.80 and 1.0% dietary phosphorus, respectively. Greatest bone weight and ash values were observed from pigs fed 0.80% dietary phosphorus when averaging both protein levels. The bone weights were significantly different (P<.01) from one another, and the bone ash values of 53.3 and 53.2% of pigs fed diets containing 0.80 and 1.0% dietary phosphorus were greater (P<.01) than 51.5% bone ash of pigs with 0.60% phosphorus in their diets. These results are in agreement with Coalson (1972) who found 0.73% dietary phosphorus adequate to support maximum skeletal development as measured by bone weight, ash, diameter, calcium and phosphorus content.

Similar response patterns were observed for bone weight and ash as dietary protein and phosphorus increased. Pigs receiving 18% protein diets had the greatest bone weight and ash values when dietary phosphorus was supplemented to the 1.0% level. The peak bone weight and ash values for pigs fed 21% protein diets were realized when 0.80% dietary phosphorus was included in the diet.

The lowest bone weight of 3.76 g occurred in pigs fed 21% protein with 0.60% phosphorus diets. This bone weight and the bone weight of 3.81 g of pigs fed 21% protein with 1.0% dietary phosphorus were significantly (P<.01) less than those of pigs on the other treatments. Increasing dietary phosphorus to the 1.0% level in the 18% protein diet resulted in pigs with an average bone weight of 4.26 g which was heavier (P<.01) than the 4.04 and 4.02 g of those pigs receiving the two lower mineral levels in diets of the same protein level. The bone weight of

4.28 g of pigs fed 21% dietary protein with 0.80% phosphorus was also greater ($P<.01$) than the 4.04 or 4.02 g weight of the above mentioned pigs.

The percent bone ash peaked at 53.7% when pigs were fed 1.0% phosphorus with 18% protein and at 54.0% when fed diets of 0.80% phosphorus with 21% protein. These bone ash values were significantly greater ($P<.01$) than those from the other treatments. Also, the 51% bone ash of pigs receiving 0.60 and 18% dietary phosphorus and protein, respectively, was lower ($P<.01$) than the 52.7 and 52.6% bone ash of pigs fed diets of 1.0% phosphorus with 21% protein and 0.80% phosphorus with 18% protein, respectively.

Trial 3. Increasing Dietary Protein and Mineral Levels With Calcium: Phosphorus Ratio At 1.25:1.

The data from the third trial are summarized in table 8. The statistical analyses of the performance parameters are shown in appendix tables 15 and 16.

Excellent performance was obtained when pigs were fed the higher protein (22%) diets. Although there were no significant differences in final weights, pigs receiving 22% protein diets had an average daily gain of 0.41 kg which was significantly greater ($P<.05$) than the 0.37 kg gain of the pigs fed 18% protein diets. An average daily feed consumption of 0.67 kg for pigs receiving 22% protein diets was less ($P<.05$) than the 0.75 kg feed consumption of pigs fed 18% protein diets. Differences were also evident in feed efficiency. Increasing dietary protein to 22% resulted in an average feed to gain ratio of 1.62 which was less ($P<.01$)

TABLE 8. EFFECT OF INCREASING DIETARY PROTEIN, CALCIUM AND PHOSPHORUS ON FEEDLOT PERFORMANCE. TRIAL 3

Item	Nutrient %	Dietary treatments (protein and mineral)						Means of main effects				
	C.P.	18			22			18	22	...	...	...
	Ca	0.75	1.0	1.25	0.75	1.0	1.25	...	...	0.75	1.0	1.25
	P	0.60	0.80	1.0	0.60	0.80	1.0	...	...	0.60	0.80	1.0
Final wt., kg		18.48	19.01	19.60	20.00	20.01	20.44	19.02	20.15	19.19	19.53	20.02
Daily gain, kg[a]		0.35	0.37	0.40	0.41	0.41	0.42	0.37	0.41	0.38	0.39	0.41
Daily feed consumption, kg[b]		0.81	0.66	0.77	0.69	0.66	0.65	0.75	0.67	0.75	0.66	0.77
Feed/gain, ratio[c]		2.29	1.79	1.96	1.71	1.61	1.55	2.01	1.62	2.00	1.70	1.76

[a]Significant ($P<.05$) protein effect (0.41 kg for 22% protein vs. 0.37 for 18% protein).

[b]Significant ($P<.05$) protein effect (0.67 kg for 22% protein vs. 0.75 kg for 18% protein).

[c]Significant ($P<.01$) protein effect (1.63 for 22% protein vs. 2.01 for 18% protein).

than the 2.01 feed to gain ratio of pigs fed 18% dietary protein. These results substantiate the protein effect on feed efficiency reported in trials 1 and 2.

When dietary calcium and phosphorus levels were increased at each of the two protein levels, the average daily gains were 0.38, 0.39 and 0.41 kg for pigs fed 0.75/0.60, 1.0/0.80 and 1.25/1.0% dietary calcium: phosphorus, respectively. Although not significant, the tendancy for gains to increase as dietary mineral levels were elevated was also observed in growing swine by Reinhard et al. (1976) in a similar protein and mineral study. The protein by mineral interaction for daily gain was not significant, but growth rates increased as dietary levels were increased, most notably in the 18% protein diet.

The higher feed consumption and feed to gain ratio of 0.81 kg and 2.29, respectively, for pigs receiving the basal diet may be attributed to feed wastage from two pens receiving this diet. Although the interaction was not significant, the feed consumption and feed to gain ratio decreased as dietary mineral levels were elevated in the 22% protein diet.

SUMMARY

This experiment consisted of three trials utilizing a total of 384 crossbred pigs to evaluate starter diets for weanling pigs of an average age of approximately 5 weeks. An 18% protein diet with 0.80% calcium and 0.60% phosphorus was used as a control diet in all three trials.

The first trial was conducted to evaluate the effects of three dietary calcium:phosphorus levels (0.80/0.60, 1.05/0.80 and 1.3/1.0%, respectively) in 18 or 21% protein diets. Feeding the 21% protein diet containing 1.3% calcium and 1.0% phosphorus resulted in pigs gaining 0.47 kg per day, which was approximately 12% faster than the gains of pigs on the other treatments. The pigs receiving diets with 21% protein consumed less feed and required significantly ($P<.01$) less feed per gain than pigs fed 18% protein diets.

No differences were observed in serum calcium concentrations. Pigs fed 18% protein diets had higher ($P<.05$) levels of serum phosphorus than those receiving 21% protein diets. Lowest serum phosphorus concentration (9.05 mg %) occurred in pigs fed 0.80% calcium, 0.60% phosphorus and 21% protein diets. The serum phosphorus level of pigs fed this diet was less ($P<.01$) than that of pigs in all other treatments except those fed diets of 18% protein, 1.3% calcium and 1.0% phosphorus.

In the second trial, 18 and 21% protein diets were formulated to contain 0.80% calcium and 0.60, 0.80 and 1.0% phosphorus. Protein and mineral supplementation appeared to have its greatest influences on feed efficiency. As observed in trial 1, pigs fed 21% protein required significantly (P<.01) less feed per unit of gain than pigs fed 18% protein diets. There were no significant differences in feed/gain among calcium and phosphorus treatments at either protein level. Daily gain improved as dietary phosphorus increased in the 21% protein diet.

Blood serum calcium levels were significantly (P<.01) different among treatments. Serum calcium decreased as dietary phosphorus increased. On the other hand, serum phosphorus increased as dietary phosphorus increased when pigs were fed 21% protein diets, but did not change when diets contained 18% protein. As reported in trial 1, the pigs receiving 18% protein diets had higher serum phosphorus than those fed 21% protein diets, however this difference was not significant.

Pigs fed 18% dietary protein had heavier (P<.01) bone weights but less (P<.05) percent bone ash than bones of pigs fed the 21% protein diet. Bone weights of pigs fed diets of 0.80% phosphorus were greater (P<.01) than those of pigs fed 0.60 or 1.0% phosphorus while percent bone ash was greater (P<.01) for pigs fed 0.80 or 1.0% phosphorus than those fed diets of 0.60% phosphorus. Peak bone values for pigs receiving 18% protein were observed at 1.0% dietary phosphorus and at the 0.80% phosphorus level when 21% protein diets were fed.

The third trial was conducted to evaluate the effects of three dietary calcium:phosphorus levels (0.75/0.60, 1.0/0.80 and 1.25/1.0%, respectively) in 18 or 22% protein diets. Protein level appeared to have the main effect on performance and feed consumption. Average daily gain was increased (P<.05), feed consumption decreased (P<.05) and feed per gain decreased (P<.01) when the dietary protein level was increased from 18 to 22%. Although not significant, average daily gain increased from 0.38 to 0.41 kg as dietary calcium and phosphorus levels were increased.

The results of this study indicate increasing protein above 18% in starter diets will significantly improve feed efficiency along with a slight increase in daily gains. Increasing dietary calcium and phosphorus in higher protein diets appears to result in slight performance improvements. It is evident that mineral supplementation for young weaned pigs beyond NRC (1973) recommendation of 0.80% calcium and 0.60% phosphorus merits consideration, particularly in higher protein diets to maintain optimum performance, serum levels of calcium and phosphorus and bone development.

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APPENDIX

TABLE 1. PROXIMATE CHEMICAL ANALYSES OF DIETS

(PERCENT AS FED). TRIAL 1

	Diets					
Protein, %	18	18	18	21	21	21
Calcium, %	0.80	1.05	1.30	0.80	1.05	1.30
Phosphorus, %	0.60	0.80	1.0	0.60	0.80	1.0
Dry matter	87.24	87.52	87.81	88.11	88.11	87.76
Protein	21.47	20.01	19.57	22.57	22.19	21.38
Crude fiber	2.61	2.40	2.37	2.05	1.91	2.30
Ether extract	2.14	1.97	1.99	2.67	2.67	2.49
Nitrogen-free extract	55.12	57.04	56.11	55.19	54.81	54.59
Ash	5.90	6.10	7.77	5.63	6.53	7.00
Calcium	0.97	1.10	1.41	0.97	1.06	1.30
Phosphorus	0.57	0.57	0.90	0.48	0.62	0.71

TABLE 2. PROXIMATE CHEMICAL ANALYSES OF DIETS

(PERCENT AS FED). TRIAL 2

	Diets					
Protein, %	18	18	18	21	21	21
Calcium, %	0.80	0.80	0.80	0.80	0.80	0.80
Phosphorus, %	0.60	0.80	1.0	0.60	0.80	1.0
Dry matter	91.90	91.86	91.77	91.95	91.90	92.29
Protein	20.25	19.13	18.66	22.56	22.75	22.41
Crude fiber	2.92	2.79	2.85	3.23	3.15	3.33
Ether extract	2.67	2.52	2.67	2.43	2.29	2.54
Nitrogen-free extract	60.15	60.83	60.76	57.12	56.93	56.54
Ash	5.91	6.59	6.83	6.61	6.78	7.47
Calcium	0.91	1.05	0.96	1.00	1.03	1.04
Phosphorus	0.55	0.71	0.87	0.51	0.84	1.00

TABLE 3. PROXIMATE CHEMICAL ANALYSES OF DIETS

(PERCENT AS FED). TRIAL 3

	Diets					
Protein, %	18	18	18	22	22	22
Calcium, %	0.75	1.0	1.25	0.75	1.0	1.25
Phosphorus, %	0.60	0.80	1.0	0.60	0.80	1.0
Dry matter	88.92	89.19	89.26	89.19	87.55	88.26
Protein	17.28	17.91	17.50	21.69	20.66	21.75
Crude fiber	3.10	2.91	3.04	4.02	2.99	3.28
Ether extract	3.25	2.83	2.93	2.69	2.56	2.61
Nitrogen-free extract	60.23	59.34	58.64	55.19	56.08	53.21
Ash	5.06	6.20	7.15	5.60	6.26	7.41
Calcium	0.79	1.20	1.48	0.91	1.15	1.35
Phosphorus	0.55	0.82	1.03	0.59	0.80	0.99

TABLE 4. COMPOSITION OF TRACE MINERAL SALT[a]

Element	Content (%)
Zinc	.8000
Cobalt	.0020
Manganese	.4000
Copper	.4800
Iron	.3300
Iodine	.0011
Sodium chloride	97.0000

[a]Trace mineral salt added as 0.4% of diet.

TABLE 5. VITAMIN-ANTIBIOTIC PREMIX FOR STARTER PIG DIETS

Compound	Vitamin or antibiotic activity supplied per kg of premix[a]
Vitamin A	1,466,667 IU
Vitamin D	146,667 IU
Vitamin E	2,200 mg
Vitamin K	880 mg
Riboflavin	1,223 mg
Pantothentic acid	4,400 mg
Niacin	7,040 mg
Choline	22,000 mg
Vitamin B_{12}	4,400 mcg
Chlortetracycline	36,667 mg
Penicillin	18,333 mg
Sulfamethazine	36,667 mg

[a]Supplied per kg of diet: vitamin A, 4400 IU; vitamin D, 440 IU; vitamin E, 6.6 mg; vitamin K, 2.6 mg; riboflavin, 3.7 mg; pantothentic acid, 13.2 mg; niacin, 21.1 mg; choline, 66 mg; vitamin B_{12}, 13.2 mcg; chlortetracycline, 110 mg; penicillin, 55 mg; and sulfamethazine, 110 mg.

TABLE 6. ANALYSIS OF VARIANCE FOR AVERAGE DAILY GAIN.

TRIAL 1

Source of variation	df	Mean squares
Protein (P)	1	.08209
Mineral (M)	2	.05133
Replication (R)	3	.29576
P x M	2	.08849
P x R	3	.01212
M x R	6	.08400
P x M x R	6	.09009
Residual	113	.04411

TABLE 7. ANALYSIS OF VARIANCE FOR FEED CONSUMPTION AND FEED-GAIN. TRIAL 1

Source of variation	df	Mean squares	
		Feed consumption	Feed-gain
Protein (P)	1	.02282	.15520**
Mineral (M)	2	.02368	.00012
Replication (R)	3	.18410	.00425
P x M	2	.05133	.00780
P x R	3	.01500	.01905
M x R	6	.04591	.00926
P x M x R	6	.05615	.00489

TABLE 8. ANALYSIS OF VARIANCE FOR SERUM CALCIUM.

TRIAL 1

Source of variation	df	Mean squares Initial	Terminal	Difference
Protein (P)	1	.00601	.09843	.14492
Mineral (M)	2	.08133	.11704	.09712
Replication (R)	3	1.11526	.13152	.51675
P x M	2	.48465	.40929	.05143
P x R	3	.84655	.13541	.54801
M x R	6	.17953	.04237	.23052
P x M x R	6	.30599	.12726	.40016
Residual	113	.32679	.21606	.40268

TABLE 9. ANALYSIS OF VARIANCE FOR SERUM PHOSPHORUS.

TRIAL 1

Source of variation	df	Mean squares Initial	Terminal	Difference
Protein (P)	1	.35448	5.73122*	3.60337
Mineral (M)	2	.07004	2.64025	3.72355
Replication (R)	3	8.98653	25.45760	47.75226
P x M	2	.01485	12.55430*	11.68081*
P x R	3	2.43874	1.54945	4.66501
M x R	6	1.06975	.54519	1.034623
P x M x R	6	.58618	1.90760	2.23626
Residual	113	.96240	1.09859	1.50432

TABLE 10. ANALYSIS OF VARIANCE FOR AVERAGE DAILY GAIN.

TRIAL 2

Source of variation	df	Mean squares
Protein (P)	1	.03240
Mineral (M)	2	.04119
Replication (R)	3	.03652
P x M	2	.01895
P x R	3	.02496
M x R	6	.06352
P x M x R	6	.03833
Residual	113	.04899

TABLE 11. ANALYSIS OF VARIANCE FOR FEED CONSUMPTION AND FEED-GAIN. TRIAL 2

Source of variation	df	Mean squares	
		Feed consumption	Feed-gain
Protein (P)	1	.05704	.13500**
Mineral (M)	2	.01030	.00153
Replication (R)	3	.01783	.02472
P x M	2	.00323	.01164*
P x R	3	.05098	.00979
M x R	6	.05273	.01827
P x M x R	6	.02429	.00198

TABLE 12. ANALYSIS OF VARIANCE FOR SERUM CALCIUM.

TRIAL 2

Source of variation	df	Mean squares Initial	Terminal	Difference
Protein (P)	1	.51462	.10142	.13433
Mineral (M)	2	.01366	2.02368**	1.96522*
Replication (R)	3	13.54113	4.40076	30.25821
P x M	2	.30835	.20160	.79240
P x R	3	.95806	.30285	1.66278
M x R	6	.12907	.34726	.43971
P x M x R	6	.77818	.47584	1.75546
Residual	113	.43670	.30623	.53301

TABLE 13. ANALYSIS OF VARIANCE FOR SERUM PHOSPHORUS.

TRIAL 2

Source of variation	df	Mean squares Initial	Terminal	Difference
Protein (P)	1	.55345	3.02693	6.27814
Mineral (M)	2	.38959	2.53813	3.50573
Replication (R)	3	12.24803	12.86167	7.51362
P x M	2	.60248	5.74780**	5.43493
P x R	3	.76868	2.90954	.96403
M x R	6	.85806	7.67982	8.46619
P x M x R	6	.97194	3.74093	3.70891
Residual	113	.84885	1.03887	1.78880

TABLE 14. ANALYSIS OF VARIANCE FOR BONE ASH AND WEIGHT. TRIAL 2

Source of variation	df	Mean squares	
		Bone ash	Bone weight
Protein (P)	1	3.21568*	5.96689×10^{5}**
Mineral (M)	2	34.17686**	5.18040×10^{5}**
Replication (R)	3	37.43360**	1.38206×10^{6}**
P x M	2	12.40823	1.10502×10^{6}
P x R	3	10.80828	2.72236×10^{6}
M x R	6	4.67133	8.43085×10^{5}
P x M x R	6	13.60554	2.27991×10^{6}

TABLE 15. ANALYSIS OF VARIANCE FOR AVERAGE DAILY GAIN.

TRIAL 3

Source of variation	df	Mean squares
Protein (P)	1	15.37731*
Mineral (M)	2	2.63937
Replication (R)	3	10.54954
P x M	2	.57501
P x R	3	6.27477
M x R	6	2.79938
P x M x R	6	1.25843
Residual	67	3.98948

TABLE 16. ANALYSIS OF VARIANCE FOR FEED CONSUMPTION AND FEED-GAIN. TRIAL 3

Source of variation	df	Mean squares Feed consumption	 Feed-gain
Protein (P)	1	.20350*	.90870**
Mineral (M)	2	.07883	.20340*
Replication (R)	3	.08119	.01974
P x M	2	.04565	.08368
P x R	3	.03345	.01975
M x R	6	.02125	.01945
P x M x R	6	.03197	.03934